

T7 primer

TTAATACGACTCACTATAGG AGACCGG AAG CTT AAG GTG CAC GGC CCA CCT GGA TCG ATC GCG GCG AGA TCT TCG GAA
Hind 3 Bgl II

-28

Met Asp Ser Lys Val Thr Ile Ile Cys Ile Arg Phe Leu Phe Trp Phe Leu Leu Cys Met Leu Ile Gly Lys Ser His Thr

GCC ACC ATG GAT AGC AAA CTC ACA ATC ATA TGC ATC AGA TTT CTC TTT TGG TTT CTT TTG CTC TGC ATG CTT ATT GGG AAG TCA CAT ACT

+1 NcoI

Glu Asp Asp Ile Ile Ala Thr Lys Asn Gly Lys Val Arg Gly Met Asn Leu Thr Val Phe Gly Gly Thr Val Thr Ala Phe Leu Gly

GAA GAT GAC ATC ATA ATT GCA ACA AAG AAT GGA AAA GTC AGA GGG ATG AAC TTG ACA GTT TTT GGT GGC AGG GTA ACA GCC TTT CTT GGA 90

40

Ile Pro Tyr Ala Gln Pro Pro Leu Gly Arg Leu Arg Phe Lys Lys Pro Gln Ser Leu Thr Lys Trp Ser Asp Ile Trp Asn Ala Thr Lys

ATT CCC TAT GCA CAG CCA CCT CTT GGT AGA CTT CGA TTC AAA AAG CCA CAG TCT CTG ACC AAG TGG TCT GAT ATT TGG AAT GCC ACA AAA 180

Eco RI

Acc I Taq I

Tyr Ala Asn Ser Cys Gln Asn Ile Asp Gln Ser Phe Pro Gly Phe His Gly Ser Glu Met Trp Asn Pro Asn Thr Asp Leu Ser Glu

TAT GCA AAT TCT TGC TGT CAG AAC ATA GAT CAA AGT TTT CCA GGC TTC CAT GGA TCA GAG ATG TGG AAC CCA AAC ACT GAC CTC AGT GAA 270

100

Asp Cys Leu Tyr Leu Asn Val Trp Ile Pro Ala Pro Lys Pro Lys Asn Ala Thr Val Leu Ile Trp Ile Tyr Gly Gly Phe Gln Thr

GAC TGT TTA TAT CTA AAT GTA TGG ATT CCA GCA CCT AAA CCA AAA AAT GCC ACT GTA TTG ATA TGG ATT TAT GGT GGT TTT CAA ACT 360

130

Gly Thr Ser Ser Leu His Val Tyr Asp Gly Lys Phe Leu Ala Arg Val Glu Arg Val Ile Val Val Ser Met Asn Tyr Arg Val Gly Ala

GGG ACA TCA TCT TTA CAT CTT TAT GAT GGC AAG TTT CTG GCT CGG GTT GAA AGA CTT ATT GTA GTG TCA ATG AAC TAT AGG GTG GGT GCC 450

Ava I

Figure 1A

160 Leu Gly Phe Leu Ala Leu Pro Gly Asn Pro Glu Ala Pro Gly Asn Met Gly Leu Phe Asp Gln Gln Leu Ala Leu Gln Trp Val Gln Lys 180
 CTA GGA TTC TTA GCT TTG CCA GGA AAT CCT GAG GCT CCA GGG AAC ATG GGT TTA TTT GAT CAA CAG TTG GCT CTT CAG TGG GTT CAA AAA 540
 190 Asn Ile Ala Ala Phe Gly Gly Asn Pro Lys Ser Val Thr Leu phe Gly Glu SER Ala Gly Ala Ala Ser Val Ser Leu His Leu Leu Ser 210
 AAT ATA GCA GCC TTT GGT GGA AAT CCT AAA AGT GTA ACT CTC TTT GGA GAA AGT GCA GGA GCA GCT TCA GTT ACC CTG CAT TTG CTT TCT 630
 220 Pro Gly Ser His Ser Leu Phe Thr Arg Ala Ile Leu Gln Ser Gly Ser Phe Asn Ala Pro Trp Ala Val Thr Ser Leu Tyr Glu Ala Arg 240
 CCT GGA ACC CAT TCA TTG TTC ACC AGA GCC ATT CTG CAA AGT GGT TCC TTT AAT GCT CCT TGG GCG GTA ACA TCT CTT TAT GAA GCT AGG 720
 CHO 250
 Asn Arg Thr Leu Asn Leu Ala Lys Leu Thr Gly Cys Ser Arg Glu Asn Glu Thr Glu Ile Ile Lys Cys Leu Arg Asn Lys Asp pro Gln 270
 AAC AGA ACG TTG AAC TTA GCT AAA TTG ACT GGT TCC TCT AGA GAG AAT GAG ACT GAA ATA ATC AAG TGT CTT AGA AAT AAA GAT CCC CAA 810
 280 CHO
 Glu Ile Leu Leu Asn Glu Ala Phe Val Val Pro Tyr Gly Thr Pro Leu Ser Val Asn Phe Gly Pro Thr Val Asp Gly Asp Phe Leu Thr 300
 GAA ATT CTT CTG AAT GAA GCA TTT GTT GTC CCC TAT GGG ACT CCT TTG TCA GTA AAC TTT GGT CCG ACC GTG GAT GGT GAT TTT CTC ACT 900
 310 Asp Met Pro Asp Ile Leu Leu Glu Leu Gly Gln Phe Lys Lys Thr Gln Ile Leu Val Gly Val Asn Lys Asp Glu Gly Thr Trp Phe Leu 330
 GAC ATG CCA GAC ATA TTA CTT GAA CTT GGA CAA TTT AAA ACC CAG ATT TTG GTG GGT GTT AAT AAA GAT GAA GGG ACA TGG TTT TTA 990
 CHO

Figure 1B

340 Val Tyr Gly Ala Pro Gly Phe Ser Lys Asp Asn Asn Ser Ile Ile Thr Arg Lys Glu Glu Gly Leu Lys Ile Phe Phe Pro Gly 360
 GTC TAT GGT GCT CCT GCC TTC AGC AAA GAT AAC AAT AGT ATC ATA ACT AGA AAA GAA TTT CAG GAA CGT TTA AAA ATA TTT TTT CCA GGA 1080
 370 Val Ser Glu Phe Gly Lys Glu Ser Ile Leu Phe His Tyr Thr Asp Trp Val Asp Asp Gln Arg Pro Glu Asn Tyr Arg Glu Ala Leu Gly 390
 Dra I SspI
 380 CTC AGT GAG TTT GGA AAG GAA TCC ATC CTT TTT CAT TAC ACA GAC TCG GTA GAT CAG AGA CCT GAA AAC TAC CGT GAG GCC TTG GGT 1170
 400 Asp Val Val Gly Asp Tyr Asn Phe Ile Cys Pro Ala Leu Glu Phe Thr Lys Lys Phe Ser Glu Trp Gly Asn Asn Ala Phe Phe Tyr Tyr 420
 Gat GTT GTT GCG GAT TAT AAT TTC ATA TCG CCT GCC TTG GAG TTC ACC AAG AAG TTC TCA GAA TGG GCA AAT AAT GCC TTT TTC TAC TAT 1260
 430 Phe Glu His Arg Ser Ser Lys Leu Pro Trp Pro Glu Trp Met Gly Val Met His Gly Tyr Glu Ile Glu Phe Val Phe Gly Leu Pro Leu 450
 TTT GAA CAC CGA TCC TCC AAA CTT CCG TGG CCA GAA TGG ATG GGA GTG ATG CAT GCC TAT GAA ATT GAA TTT GTC TTT GGT TTA CCT CTG 1350
 CHO 460 470 480
 Glu Arg Arg Asp Asn Tyr Thr Lys Ala Glu Glu Ile Leu Ser Arg Ser Ile Val Lys Arg Trp Ala Asn Phe Ala Lys Tyr Gly Asn Pro
 GAA AGA AGA GAT AAT TAC ACA AAA GCC GAG GAA AAT TTG AGT AGA TCC ATA GTG AAA CGG TGG GCA AAT TTT GCA AAA TAT GCG AAT CCA 1440
 CHO 490 500 510
 Asn Glu Thr Gln Asn Asn Ser Thr Ser Trp Pro Val Phe Lys Ser Thr Glu Gln Lys Tyr Tyr Leu Thr Leu Asn Thr Glu Ser Thr Arg Ile
 AAT GAG ACT CAG AAC AAT AGC ACA ACC TGG CCT GTC TTC AAA AGC ACT GAA CAA AAA TAT CTA ACC TTG AAT ACA GAG TCA ACA AGA ATA 1530
 Hinc II

Figure 1C

Met Thr Lys Leu Arg Ala Gln Cys Arg Phe Thr Ser Phe Phe Pro Lys Val Leu Glu Met Thr Gly Asn Ile Asp Glu Ala Glu	520	530	540
ATG ACG AAA CTA CGT GCT CAA CAA TGT CGA TTC TGG ACA TCA TTT TTT CCA AAA GTC TTG GAA ATG ACA GGA AAT ATT GAT GAA GCA GAA			
	<u>Taq I</u>		
Trp Glu Trp Lys Ala Gly Phe His Arg Trp Asn Asn Tyr Met Met Asp Trp Lys Asn Gln Phe Asn Asp Tyr Thr Ser Lys Lys Glu Ser		560	570
TGG GAG TGG AAA GCA GGA TTC CAT CGC TGG AAC AAT TAC ATG ATG GAC TGG AAA AAT CAA TTT AAC GAT TAC ACT ACC AAG AAA GAA AGT			
		<u>Ssp I</u>	
			1710
574			
Cys Val Gly Leu ***			
TGT GTG GGT CTC TAA TTA ATA GAT CTG TCA TGA TGA TCA TTG CAA TTG GAT CCA TAT ATA GGG CCC TATT CTATAGTGTACCTAAAT			
	<u>Ase I</u>	<u>Bgl II</u>	
		Bam HI, Eco01091, Apa I	Sp6 primer

Figure 1D

5/11

EDDIIIATKNGKVRGMNLTVEGGTVTAFLGIPYAQPPLGRLRFKKPQSLTK
WSDIWNATKYANSCCQNIDQSFPGFHGSEMWNPNTDLSEDCLYLNWIPAP
KPKNATVLIWIYGGGFQTGTSSLHVYDGKFLARVERVIVVSMNYRVGALGF
LALPGNPEAPGNMGLFDQQLALQWVQKNIAAFGGNPKSVTLFGESAGAASV
SLHLLSPGSHSLFTRAILQSGSFNAPWAVTSLYEARNRTLNLAKLTGCSRE
NETEIIKCLRNDKDPQEILLNEAFVVPYGTPLSVNFGPTVDGDFLTDMPDIL
LELGQFKKTQILVGVNKDEGTAFLVYGAPGFSKDNNSIITRKEFQEGLKIF
FPGVSEFGKESILFHYTDWVDDQRPENYREALGDVVGDYNFICPALEFTKK
FSEWGNNAFFYYFEHRSSKLPWPEWMGVMHGYEIEFVFGPLPLERRDNYTKA
EEILSR
SIVKRWANFAKYGNPNETQNNSTSWPVFKSTEQKYLT
LNTESTRI
MTKLRAQQCRFWTSFFPKVLEMTGNIDEAEWEWKAGFHRWNNYMMDWKNQF
NDYTSKKESCVGL

09748739-060101
TOTFO90"6E284650

Figure 2

1 tactgaatgt cagtgcagtc caatttacag gctggagcag cagetgcate ctgcatttcc
 61 ccgaagtatt acatgatttt cactccttgc aaactttacc atctttgttg cagagaatcg
 121 gaaatcaata tgcatagcaa agtcacaatc atatgcatca gattttctctt ttggtttctt
 181 ttgctctgca tgcttattgg gaagtcacat actgaagatg acatcataat tgcaacaaag
 241 aatggaaaag tcagagggat gaacttgaca gtttttgggtg gcacggtaac agcctttctt
 301 ggaattccct atgcacagcc acctcttggg agacttcgat tcaaaaagcc acagtctctg
 361 accaagtggg ctgatatttg gaatgccaca aaatatgcaa attcttggctg tcagaacata
 421 gatcaaagtt ttccaggctt ccatggatca gagatgtgga acccaaacac tgacctcagt
 481 gaagactggt tatatctaaa tgtatggatt ccagcaccta aacaaaaaaa tgccactgta
 541 ttgatatgga tttatgggtg tggttttcaa actggaacat catctttaca tgtttatgat
 601 ggcaagtttc tggctcgggt tgaaagagtt attgtagtgt caatgaacta taggggtgggt
 661 gccctaggat tcttagcttt gccaggaaat cctgaggctc cagggaacat gggtttattt
 721 gatcaacagt tggctcttca gtgggttcaa aaaaatatag cagccttttg tggaaatcct
 781 aaaagtgtaa ctctcttttg agaaagtgca ggagcagctt cagttagcct gcatttgctt
 841 tctcctggaa gccattcatt gttcaccaga gccattctgc aaagtggatc ctttaatgct
 901 ccttggggcg taacatctct ttatgaagct aggaacagaa cgttgaactt agctaaattg
 961 actggttgct ctagagagaa tgagactgaa ataatcaagt gtcttagaaa taaagatccc
 1021 caagaaattc tcttgaatga agcatttggt gtcccctatg ggactccttt gtcagtaaac
 1081 tttggtecca ccgtggatgg tgattttctc actgacatgc cagacatatt acttgaactt
 1141 ggacaattta aaaaaaccca gattttgggt ggtgttaata aagatgaagg gacagctttt
 1201 ttagtctatg gtgctcctgg cttcagcaaa gataacaata gtatcataac tagaaaagaa
 1261 tttcaggaag gtttaaaaat attttttcca ggagtgagtg agtttggaag ggaatccatc
 1321 ctttttcatt acacagactg ggtagatgat cagagacctg aaaactaccg tgaggccttg
 1381 ggtgatgttg ttggggatta taatttcata tgccctgcct tggagtccac caagaagttc
 1441 tcagaatggg gaaataatgc ctttttctac tattttgaac accgatcctc caaacttccg
 1501 tggccagaat ggatgggagt gatgcatggc tatgaaattg aatttgtctt tggtttacct
 1561 ctggaaagaa gagataatta cacaaaagcc gaggaattt tgagttagatc catagtgaag
 1621 cgggtgggcaa attttgcaaa atatgggaat ccaaatgaga ctcagaacaa tagcacaagc
 1681 tggcctgtct tcaaaagcac tgaacaaaaa tatctaacct tgaatacaga gtcaacaaga
 1741 ataatgacga aactacgtgc tcaacaatgt cgattctgga catcattttt tccaaaagtc
 1801 ttggaatga caggaaatat tgatgaagca gaatgggagt ggaaagcagg attccatcgc
 1861 tggaacaatt acatgatgga ctggaaaaat caatttaacg attacactag caagaaagaa
 1921 agttgtgtgg gtctctaatt aatagattta ccctttatag aacatatttt ccttttagatc
 1981 aaggcaaaaa tatcaggagc ttttttacac acctactaaa aaagttatta tgtagctgaa

Figure 3A

2041 acaaaaatgc cagaaggata atattgattc ctcacatctt taacttagta ttttacctag
2101 catttcaaaa cccaaatggc tagaacatgt ttaattaaat ttcacaatat aaagttctac
2161 agttaattat gtgcatatta aaacaatggc ctggttcaat ttctttcttt cottaataaa
2221 ttttaagtttt ttccccccaa aattatcagt gctctgcttt tagtcacgtg tattttcatt
2281 accactcgta aaaaggtatc ttttttaaata gaattaaata ttgaaacact gtacaccata
2341 gtttacaata ttatgtttcc taattaaaat aagaattgaa tgtcaatatg agatattaaa
2401 ataagcacag aaaatc

Figure 3B

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	1	10	20	30
HUMAN WILD-TYPE BChE	EDDIIIATKN	GKVRGMNLTV	FGGTVTAFLG	
HUMAN A VARIANT BChE	-----	-----	-----	
HUMAN J VARIANT BChE	-----	-----	-----	
HUMAN K VARIANT BChE	-----	-----	-----	
RAT BChE	EEDVIITTKT	GRVRGLSMPI	LG GTVTAFLG	
CAT BChE	EEDIIITTKN	GKVRGMNLPV	LD GTVTAFLG	
HORSE BChE	EEDIIITTKN	GKVRGMNLPV	LG GTVTAFLG	

	40	50	60	70	80	90	100
HUMAN WT	IPYAQPPLGR	LRFKKPQSLT	KWSDIWNATK	YANSCCQNI	DSFPGFHGSE	MWNPNTDLSE	DCLYLNWVWP
HUMAN A	-----	-----	-----	-----G	-----	-----	-----
HUMAN J	-----	-----	-----	-----	-----	-----	-----
HUMAN K	-----	-----	-----	-----	-----	-----	-----
RAT	IPYAQPPLGS	LRFKKPQPLN	KWPDVYNATK	YANSCYQNI	QAFPGFQGSE	MWNPNTNLSE	DCLYLNWVWP
CAT	IPYAQPPLGR	LRFKKPQFLT	KWSDIWNATK	YANSCYQNA	DSFPGFPGSE	MWNPNTDLSE	DCLYLNWVWP
HORSE	IPYAQPPLGR	LRFKKPQSLT	KWSNIWNATK	YANSCYQNT	DSFPGFLGSE	MWNPNTLSE	DCLYLNWVWP

	110	120	130	140	150	160	170
HUMAN WT	APKPKNATVL	IWIYGGGFQT	GTSSLHVDYD	KFLARVERVI	VVSMNYRVGA	LGFLALPGNP	EAPGNMGLFD
HUMAN A	-----	-----	-----	-----	-----	-----	-----
HUMAN J	-----	-----	-----	-----	-----	-----	-----
HUMAN K	-----	-----	-----	-----	-----	-----	-----
RAT	VPKPKNATVM	VWVYGGGFQT	GTSSLPVYD	KFLTRVERVI	VVSMNYRVGA	LGFLAFPGNS	EAPGNMGLFD
CAT	TPKPKNATVM	IWIYGGGFQT	GTSSLPVYD	KFLARVERVI	VVSMNYRVGA	LGFLALPGNP	EVPGNMGLFD
HORSE	APKPKNATVM	IWIYGGGFQT	GTSSLPVYD	KFLARVERVI	VVSMNYRVGA	LGFLALSEN	EAPGNMGLFD

	180	190	200	210	220	230	240
HUMAN WT	QQLALQWVQK	NIAAFGGNFK	SVTLFGESAG	AASVSLHLLS	PGSHSLFTRA	ILQSGSFNAP	WAVTSLYEAR
HUMAN A	-----	-----	-----	-----	-----	-----	-----
HUMAN J	-----	-----	-----	-----	-----	-----	-----
HUMAN K	-----	-----	-----	-----	-----	-----	-----
RAT	QQLALQWIQR	NIAAFGGNPK	SVTLFGESAG	AASVSLHLLC	PQSYPLFTRA	ILESGSSNAP	WAVKHPEEAR
CAT	QQLALQWVQK	NIAAFGGNPK	SVTLFGESAG	AGSVSLHLLS	PRSQPLFTRA	ILQSGSSNAP	WAVMSLDEAK
HORSE	QQLALQWVQK	NIAAFGGNPR	SVTLFGESAG	AASVSLHLLS	PRSQPLFTRA	ILQSGSSNAP	WAVTSLYEAR

	250	260	270	280	290	300	310
HUMAN WT	NRTLNLAKLT	GCSRENETE	IKCLRNKDPQ	EILLNEAFV	PYGTPLSVNF	GPTVDGDFLT	DMPDILLELG
HUMAN A	-----	-----	-----	-----	-----	-----	-----
HUMAN J	-----	-----	-----	-----	-----	-----	-----
HUMAN K	-----	-----	-----	-----	-----	-----	-----
RAT	NRTLTLAKFI	GCSKENEKEI	ITCLRSKDPQ	EILLNEKLVL	PSDSIRSINF	GPTVDGDFLT	DMPHTLLQLG
CAT	NRTLTLAKFI	GCSKENDTEI	IKCLRNKDPQ	EILLNELLV	PSDTLLSVNF	GPVVDGDFLT	DMPDTLLQLG
HORSE	NRTLTLAKRM	GCSRDNEM	IKCLRDKDPQ	EILLNEFV	PYDTLLSVNF	GPTVDGDFLT	DMPDTLLQLG

Figure 4A

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	320	330	340	350	360	370	380
HUMAN WT	QFKKTQILVG	VNKDEGTAF	LYGAPGFSKD	NNSIITRKEF	QEGDKIFFPG	VSEFGKESIL	FHYTDWVDDQ
HUMAN A	-----	-----	-----	-----	-----	-----	-----
HUMAN J	-----	-----	-----	-----	-----	-----	-----
HUMAN K	-----	-----	-----	-----	-----	-----	-----
RAT	KVKTAQILVG	VNKDEGTAF	LYGAPGFSKD	NDSLITRREF	QEGLNMYFPG	VSSLGKEAIL	FYYVDWLGDQ
CAT	QFKKTQILVG	VNKDEGTAF	LYGAPGFSKD	NDSIITRKEF	QEGDKIYFPG	VSEFGREAIL	FYYVDLLDDQ
HORSE	QFKRTQILVG	VNKDEGTAF	LYGAPGFSKD	NNSIITRKEF	QEGDKIFFPR	VSEFGRESIL	FHYMDWLDDQ

	390	400	410	420	430	440	450
HUMAN WT	RPENYREALG	DVVGDFNFIC	PALEFTKKFS	EWGNNAFFYY	FEHRSSKLPW	PEWMGVMHGY	EIEFVFGGLPL
HUMAN A	-----	-----	-----	-----	-----	-----	-----
HUMAN J	-----	-----	-----	-----	-----	-----	-----
HUMAN K	-----	-----	-----	-----	-----	-----	-----
RAT	TPEVYREAFD	DIIGDYNIC	PALEFTKKFA	ELEINAFYY	FEHRSSKLPW	PEWMGVMHGY	EIEFVFGGLPL
CAT	RAEKYREALD	DVLGDYNIC	PALEFTTKFS	ELGNNAFFYY	FEHRSSQLPW	PEWMGVMHGY	EIEFVFGGLPL
HORSE	RAENYREALD	DVVGDFNFIC	PALEFTRKFS	ELGNDAFFYY	FEHRSTKLPW	PEWMGVMHGY	EIEFVFGGLPL

	460	470	480	490	500	510	520
HUMAN WT	ERRDNYTKAE	EILSRIVKR	WANFAKYGNP	NETQNNSTSW	PVFKSTEQKY	LTLNTESTRI	MTKLRAQQCR
HUMAN A	-----	-----	-----	-----	-----	-----	-----
HUMAN J	-----	-----	-----	-----	-----	-----	-----
HUMAN K	-----	-----	-----	-----	-----	-----	-----
RAT	ERRVNYTRAE	EIFRSIMKT	WANFAKYGHP	NGTQGNSTVW	PVFTSTEQKY	LTLNTEKSKI	NSKLRAPOCQ
CAT	ERRVNYTRAE	EILSRIMNY	WANFAKYGNP	NGTQNNSTRW	PAFRSTDQKY	LTLNATESPKV	YTKLRAQQCR
HORSE	ERRVNYTRAE	EILSRIMKR	WANFAKYGNP	NGTQNNSTRW	PVFKSTEQKY	LTLNATESPKV	YTKLRAQQCR

	530	540	550	560	570	574
HUMAN WT	FWTSFFPKVL	EMTGNIDEAE	WEWKAGFHRW	NNYMDWKNQ	FNDYTSKKES	CVGL
HUMAN A	-----	-----	-----	-----	-----	----
HUMAN J	-----	-----	-----	-----	-----	----
HUMAN K	-----	-----	-----	-----	-----	----
RAT	FWRLFFPKVL	EITGDIDERE	QEWKAGFHRW	SNYMDWKNQ	FNDYTSKKES	CTDL
CAT	FWTLFFPKVL	EMTGNIDEAE	REWRAGFYRW	NNYMDWKNQ	FNDYTSKKES	CAGL
HORSE	FWTLFFPKVL	ELTGNIDEAE	REWKAGFHRW	NNYMDWKNQ	FNDYTSKKES	CSDF

Figure 4B

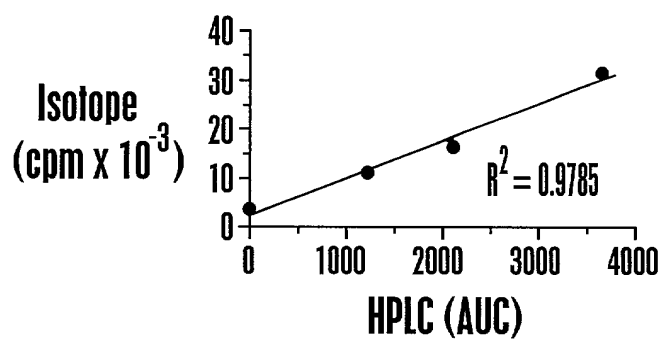


Figure 5A

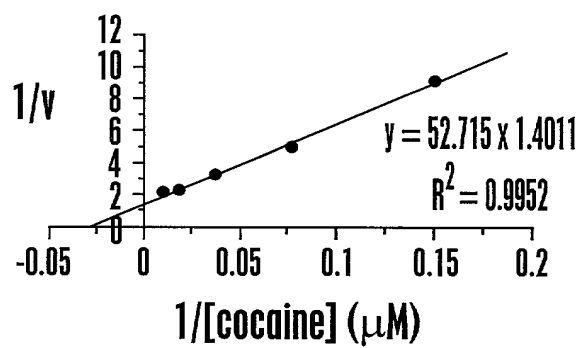


Figure 5B

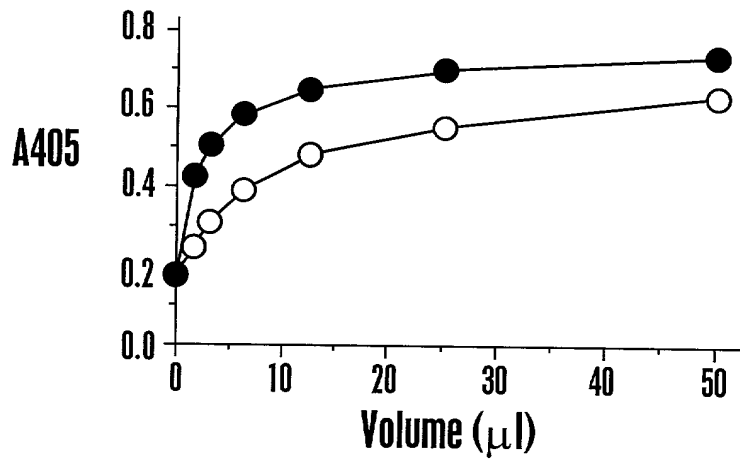


Figure 6

Figure 7

